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13. ABSTRACT (Maximum 200 Words) In our laboratory we have developed lentiviral vectors for use in anti-breast cancer gene therapy. Our specific goal for this project is to evaluate the feasibility of using lentiviral vectors that express anti-HER-2/neu antisense or ribozymes for the treatment of breast cancer. The advantage of lentiviral vectors over other vectors is that lentiviral vectors can transduce human cells with great efficiency. We have demonstrated that HIV-based lentiviral vectors can transduce a variety of human cell types with up to 99% efficiency, as measured by FACS analysis of GFP expression in vector-transduced cells. For example, Sup T1 cells can be routinely transduced with greater than 99% efficiency. This expression is stable with cells expressing high levels of GFP for greater than 60 days in culture. Primary human CD4+ T cells are routinely transduced with up to 95% efficiency. Strikingly, CD34+ hematopoietic stem cells are transduced with up to 90% efficiency in NOD/SCID mice, as assayed by measuring GFP expression in the SCID repopulating cell population (SRC). Finally, breast cancer cells can be efficiently transduced with a GFP expressing lentiviral vector. Constructs expressing an anti-HER-2/neu antisense/ribozyme payload have been constructed and are ready for testing in breast cancer cell lines in vitro and in vivo.				
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Introduction:

The overall goal of this project is to develop novel lentiviral vectors for cancer gene therapy. Our specific goal for this project is to evaluate the feasibility of using lentiviral vectors that express anti-HER-2/neu antisense or ribozymes for the treatment of breast cancer. The advantage of lentiviral vectors over other vectors is that these vectors can transduce human cells with great efficiency. The goals for this project are as follows: (1) construct lentiviral vectors and demonstrate efficient transduction of human cells, particularly human breast cancer cells and (2) test the anti-Her2/neu antisense/ribozyme expressing vectors for their ability to inhibit Her2/neu expression in tumor cell lines in vitro and in small animal models.

Research Accomplishments to date:

Construction of basic vector and helper constructs for production of high titer HIV-based lentiviral vectors

HIV-vector constructs based on the original vectors designed by Dropulic *et al* (PNAS USA 93:11103-11108, 1996) were optimized to increase their transduction efficiency. The salient features of the vector are shown below in figure 1.

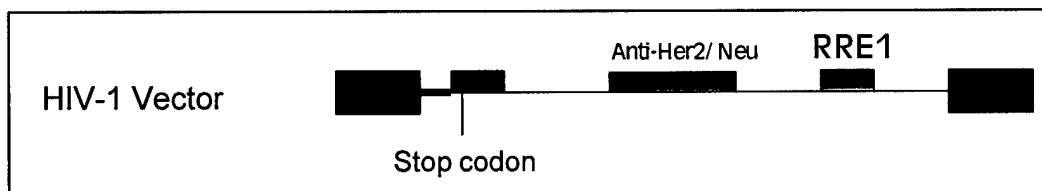


Figure 1. Salient features of HIV vectors. The antisense or ribozyme anti-Her2/neu payload (blue) is inserted upstream of the RRE (green) element that is flanked by two HIV-LTRs (grey). The packaging sequence (purple) contains a stop codon 41 nucleotides from the start of transcription to prevent translation of a Gag sequence that would be immunogenic since the first known CTL epitope for HIV gag is downstream of the stop-codon modified site. An optional green fluorescent protein (GFP) gene is present downstream of the RRE sequence (not shown).

New VIRPAC helper production system for production of high titer HIV-based vectors

A new helper production system was established for high efficiency HIV-based vector production. The production system uses a VIRPAC helper plasmid that codes for the HIV structural proteins and the VSV-G envelope protein. Therefore, HIV-based vectors have a broad tropism for many primary and tumorigenic cell types, including breast cancer cells. Using NUNC cell factories, we can produce in excess of 10^{10} transducing vector units per cell factory.

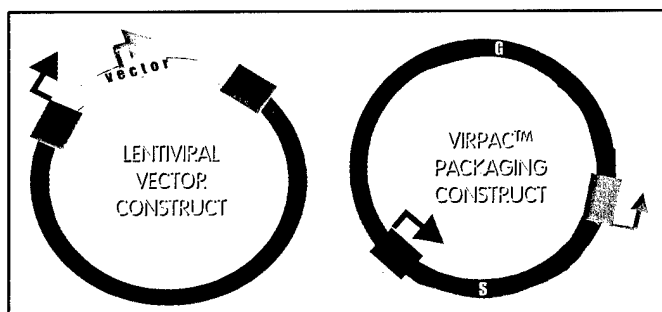


Figure 2. Schematic representation of the vector and the VIRPAC packaging constructs used for transient production of the HIV-vector in 293T cells. On the left, the vector construct shows two green LTRs with a yellow backbone. The red arrow shows the start of genomic vector RNA transcription while the blue arrow shows the start of the anti-HER2/neu antisense RNA transcription. On the right, the VIRPAC helper construct shows the S coding sequence in blue. This sequence codes for the HIV structural and enzymatic proteins while the purple G sequence codes for the VSV-G envelope protein that confers broad tropism to the HIV vector.

High efficiency transduction of a variety of primary human cell types and breast cancer cell lines

Conditions for efficient transduction of a variety of primary human cells and tumor cell lines were optimized. We found that many primary human cells could be stably transduced with greater than 90% efficiency, as measured by GFP expression. For example, 92.8% of primary human CD4⁺ T cells was transduced with a GFP-expressing HIV vector, as determined by FACS analysis of GFP positive cells.

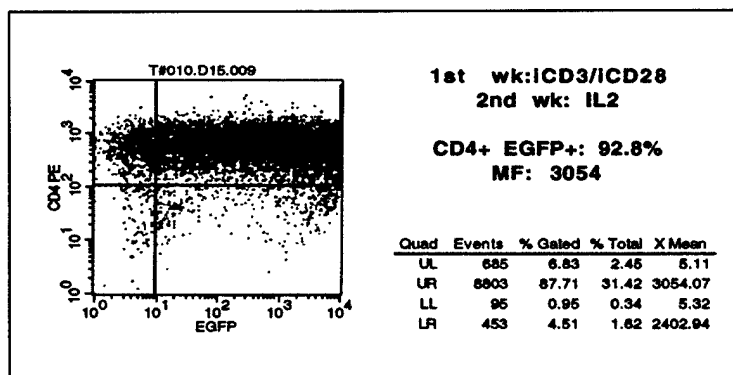


Figure 3. An example of high efficiency transduction of primary human cells by an HIV-based vector. CD4⁺ T cells were isolated from a donor and incubated with the vector at a multiplicity of infection of 20, cultured in media containing iCD3/28 during the first week and IL-2 in the second week and then analyzed by FACS. Over 90% of the cells were GFP positive, demonstrating that at least 90% of the cells stably contained the vector. Significantly, no vector-related toxic effects were observed.

We have also worked on transduction methods for other primary human cell types. The CD34⁺ stem cell is an important cell type for human gene therapy. We are able to efficiently transduce CD34⁺ stem cells derived from human cord blood with the HIV-based lentiviral vector. Strikingly, transduction efficiencies of over 90% were detected eight weeks after transplantation of the transduced cells in NOD/SCID mice.

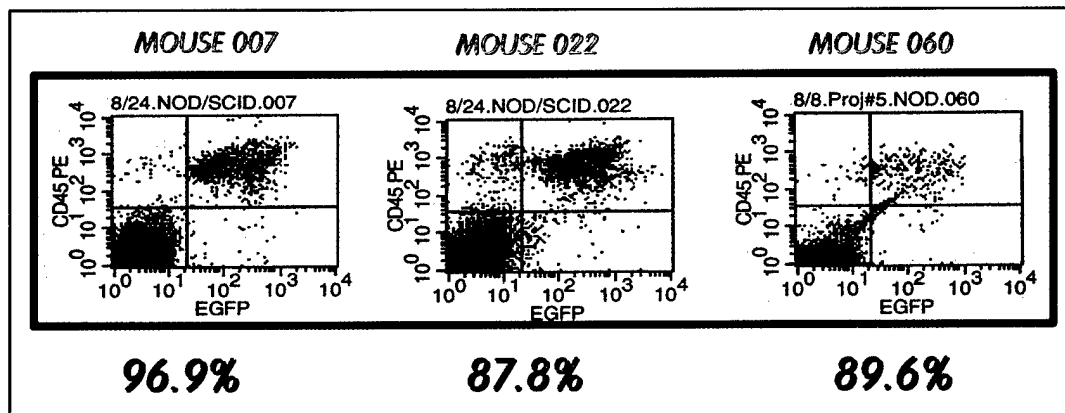


Figure 4. Efficient transduction of human CD34⁺ cells with an HIV-vector that expresses GFP. Cells were transduced with vector in the presence of a cytokine cocktail and then transplanted into NOD/SCID mice. Blood and bone marrow cells were harvested from the mice 8 weeks after the transplant and analyzed by FACS. The three animals shown above were one complete group. On average, over 90% of the human cells expressed GFP.

Conditions for efficient transduction of breast cancer cells were optimized. Varying multiplicities of infection of HIV-based vectors were used to determine which MOI produced the highest level of transduction without adverse effects.

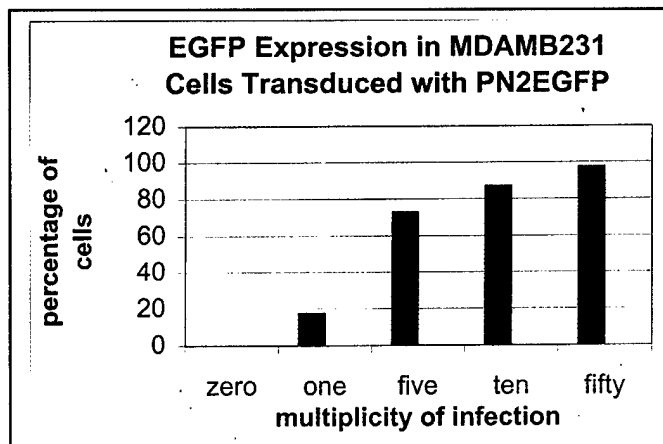


Figure 5. High efficiency transduction of the human breast cancer cell line MDA-MB-231 by the HIV-based vector. MDA-MB-231 cells were incubated with vector at varying multiplicity of infections and then analyzed by FACS analysis 2 days after transduction. Significantly, over 97% transduction efficiency of this breast cancer cell line was achieved by using an MOI of 50 of the HIV-based lentiviral vector. Similar results were obtained with the MCF-7 breast cancer cell line. HER-2-overexpressing breast cancer cell lines are being tested.

Summary

We have developed a powerful vector system for efficient transduction of human breast cancer cells. This vector system has the potential to be used in synergy with several promising anti-breast cancer payload genes that are currently being evaluated in the scientific community. We will now validate the anti-cancer potential for this HIV-vector system by testing the vector with a candidate anti-breast cancer payload. We have developed anti-HER2/neu antisense HIV-vectors that will be tested in breast cancer cells for (1) their ability to down-regulate HER2/neu expression in vitro and (2) their ability to decrease the tumorigenicity of HER-2-expressing breast cancer cells in small animal models.

Appendix

List of Key Research Accomplishments

1. Development of a vector and helper system for high efficiency production of HIV vectors
2. Achieved high efficiency transduction of primary human cells and two breast cancer cell lines
3. The HIV-vector construct containing the anti-HER2/neu antisense payload is completed

Reportable Outcomes

Either the Johns Hopkins University or VIRxSYS Corporation has previously submitted all intellectual property pertaining to the vector system to the USPTO. No new IP was developed during the course of the current work.